African Journal of Biotechnology Vol. 4 (9), pp. 960-962, September 2005
Available online at http://www.academicjournals.org/AJBk
ISSN 1684–5315 © 2005 Academic Journals

Full Length Research Paper

Effect of mineral concentration of culture media without growth substances on the callogenesis of *Atriplex halimus* L

Héla El Ferchichi Ouarda

Institut National de Recherches en Génie Rural, Eaux et Forêts, BP 10, Ariana 2080. Tunisie. Tel: +216 97 504 166. E-mail:hfo@topnet.tn.

Accepted 11 August, 2005

The studies on the in vitro culture of the *Atriplex halimus*, a fooder plant of arid zones of Tunisia, have shown an induction of the callogenesis on intact seedlings cultivated on medium without growth substances. Two media, which differ only by the nature and the concentration of macroelements, were tested. Assays of callogenesis induction were made on media with 1/2, 1/5, 1/10, 1/50, and 1/100 of the usual concentration of macroelements. Murashige and Skoog (MS) diluted at 1/2 and Gamborg (G) diluted at 1/10 showed good callogenesis effects. The control of the callogenesis seems to be possible by the macroelements, probably the mechanism involved the cellular membrane correlatively with the rate of endogenous hormones.

Key words: *Atriplex halimus, in vitro* culture, callogenesis, mineral elements.

INTRODUCTION

Atriplex are plants adapted to the arid and hot environments. In Tunisia, fields of *Atriplex* are distributed in the arid areas which receive between 200 and 400 mm of rains per year, where the ecological imbalance tends towards desertification. *Atriplex halimus* L is an indigenous species and its resistance to harsh environment can be a rational for its exploitation in lands with high salts content on which few species can develop, where the natural vegetation is profoundly degraded, and where the agricultural production is very irregular (Ben ayed, 1975). Indeed, *Atriplex* constitute the base of new agronomy for the arid areas (Ben Rebiha, 1987; Le Houérou, 1992; Haddioui and Baaziz, 2001; Abbad et al., 2003). These areas mostly show a high fodder deficit during the difficult seasons (dryness).

In vitro culture of *A. halimus* was undertaken with the aim of obtaining homogeneous and effective individuals quickly. The vigorous seedlings chosen can be used as heads of clones and cultivated in the steppe areas for repopulation the arid and pre-desert regions in a program against desertification. However, the seedlings obtained after germination and placed on culture media without growth hormones, develop callus. Thus, we attempt to find the mineral and optimal conditions for this callogenesis.

MATERIALS AND METHODS

Seeds of *A. halimus* were collected from a natural habitat in Métouia area of Gabès (South of Tunisia). The *in vitro* techniques used were as described by (Gautheret, 1959). Seeds were sterilized for 45 min with a 4% solution of calcium hypochlorithe followed by three 5 min sterile distilled water rinses (Ammar, 1977). The period of 45 min of sterilization in 4% solution of calcium hypochlorithe, was the most effective. Indeed, infection was reduced. After 4 weeks on MS medium, 95% of seedlings were free of visible contamination. The duration of 20 or 30 min was insufficient as these led to 87.5 and 66.7% infection, respectively.

The seeds were placed to germinate in Petri dishes with saturated filter paper with distilled water at 25 ± 2°C, under a 16 h photoperiod (25 $E.S^{-1}m^{-2}$). Seedlings of 1.5 and 2 cm were transferred on two different solid culture media deprived of growth regulators. These were Murashige and Skoog (MS) and Gamborg (G) media (Table 1; Gautheret, 1959). The culture media differ only by nature and concentration of macronutrients. The media were used as follows: MS, MS/2, MS/5, MS/10, G/10, G/20), G/50 and G/100. All mediums contained 20 g/l sucrose, 2 ml/l of Morel vitamins (Table 2), 5 ml/l of iron EDTA and 8 g/l of agar. The media pH was adjusted to 5.8 with 0.1 N HCl or NaOH after the agar was

Table 1. Composition of mineral solutions of Murashige and Skoog (MS) and Gamborg (G).

Macronutrients	MS (mg/l)	G (mg/l)
$NH_4 NO_3$	1650	_
$CaCl_2 2H_2O$	440	150
$MgSO_4 7H_2O$	370	250
KNO_3	1900	2500
KH_2PO_4	170	_
$(NH_4)_2 SO_4$	_	134
$NaH_2PO_4 2H_2O$	_	150

Table 2. Composition of the solution of Morel vitamins (Morel, 1968).

Vitamin	MS (mg/100 ml)	G (mg/100 ml)
Meso-inositol	5000	5000
Pantothenate de calcium	50	50
Acide nicotinique	50	50
Pyridoxine	50	50
Thiamine	50	50
Biotine	0.5	0.5

dissolved and prior to autoclaving at 120°C for 20 min. No growth regulators were added to the media. Seedlings (1.5 to 2 cm) obtained after germination and comprised 2 cotyledons, were inoculated on culture media. The cultures were incubated at the same conditions of temperature and light used for germination. Results were recorded for 24 explants per medium after 4 weeks in culture.

The test X^2 was used to estimate the importance of the distances between observed frequencies (f_0) inside random samples and expected theoretical frequencies (fe). The value of X^2 was calculated by means of the following formula (Schwartz, 1993): $X^2 = \Sigma(f_0 - f_e)^2 / f_e$. If all the observed frequencies were equal to the hoped frequencies, this sum would be equal to 0. Thus 0 was the ideal value which should take the value of X^2 when the null hypothesis is true.

RESULTS AND DISCUSSION

The results showed that the earliest induction of callogenesis ocured on the third day of culture. This callogenesis was strictly dependent on the type and concentration of macronutrients in the medium (Tables 1 and 3). Large exuberance of callogenesis was observed on MS/2 and G/10 mediums deprived of growth substances. The frequency of callus was 81.81% on MS/2 and 76.47% on G/10 (Table 4). The calluses induced on MS/2 and G/10 media were yellow and compact, while they were brown and looser on the most diluted mediums.

Table 3. Principal characteristics of Murashige and Skoog (MS) and Gamborg (G) media.

Medium	Total ionic concentrations (mM)	Dominant ions	Forms of nitrogen (mM)
MS	93.3	NO_3^-, NH_4^+, K^+	$NO_3^-/NH_4^+ = 12.5$
G	60.2	NO_3^-, K^+	$NO_3^-/NH_4^+ = 1.91$

Organogenesis with a high rate of axillary ramification could be observed on MS. The endogenous substances and especially the growth regulators are in sufficient quantity to allow normal development of the axillary buds (Elloumi, 1989). These ramifications increased the number of leaves. The principal shoot was thick, while the root system was very ramified and superficial.

Purple pigments was also observed on callus developed on G/20, G/50 and G/100. This production of purple pigments can be influenced by several physiological factors (Gautheret, 1959). The light and deficiency of macronutrients may be two factors allowing the apparition of these purple pigments. These purple pigments also invade the root system which proliferated especially in diluted culture media. Thickening of the roots was equally observed on the diluted media. That may be explained by the fact that the media were impoverished of potassium iodide (Zryd, 1988). On Gamborg media we did not observed important axillary ramification.

The calculated X^2 which was equal to 64.68 and the critical value of the table which was equal to 14.067 for $\alpha = 0.05$ and dl = 7. The X^2 (64.68) is superior to the critical value (14.067). Consequently the null hypothesis was rejected indicating that the callogenesis was significantly made in a different way according to the culture medium. This phenomenon was reproducible.

This study has demonstrated that the type and concentration of macronutrients have important effects on the callus in *A. halimus*. The highest percentage of callus occured on MS/2 and G/10 media without growth regulators. The calli obtained were heterogeneous. However, there were young seedlings which showed a normal development on diluted media like MS/5 and MS/10. There is a high potentiality of axillary ramifications of young seedlings of *A. halimus* on MS medium which is rich in K^+, NO_3^-, $NH4^+$ (Table 3). Rooting increased when the medium was diluted and the rate of KI decreases (Zryd, 1988). The control of the callogenesis depends on the choice of the macroelements used during the development of the seedlings.

This experimental system, which does not require exogenic hormones, is particularly attractive. The use of the macroelements, as factors of activation of morphogenetic phenomena, would thus allow for the

Table 4. Influence of MS and G mediums on callus development in *A. halimus*.

Seedlings	MS	MS/2	MS/5	MS/10	G/10	G/20	G/50	G/100	Total
Seedlings with callus	13	20	08	02	19	08	02	03	75
Seedlings without callus	11	04	16	22	05	16	22	21	117
Total	24	24	24	24	24	24	24	24	192

study of macronutrients under conditions similar to the natural conditions. This would avoid possible interferences with other physiological processes caused by the addition of the exogenic hormones. The balance of elements which are favorable for callogenesis or organogenesis in *A. halimus* would be investigated in further studies.

REFERENCES

Abbad A, Cherkaoui M, Benchaabane A (2003). Morphology and allozyme variability of three natural populations of *Atriplex halimus* L. Ecol. Mediterranea. 29:99-109.

Ammar S (1977). Multiplication végétative du palmier dattier (*Phoenix dactylifera* L), par la culture de tissus de jeunes plantes issues de semis. C.R Acad. Sci. Paris. 284: 1789–1791.

Ben Ayed H (1975). Un outil pour l'aménagement pastoral des zones arides : les collections et les vergers à graines de plantes fourragères à El Grin. Bull infor INRF (Tunisie). 19:1-9.

Ben Rebiha F (1987). Contribution à l'étude de la germination de quelques espèces d'*Atriplex* locales et introduites. Thèse de spécialité. Institut National Agronomique d'Algérie : 1-119.

Elloumi N (1989). Etude préliminaire de la biologie et des possibilités de multiplication végétative in vitro du Jojoba. D.E.A. Faculté des Sciences de Tunis.

Gautheret (1959). La culture des tissus végétaux : techniques et réalisations. Masson Edit.

Haddioui A, Baaziz M (2001). Genetic diversity of natural populations of *Atriplex halimus* L. in Morocco: An isoenzyme-based overview. Euphytica. 121: 99-106.

Le Houérou HN (1992). The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: A review Agrofor Syst. 18:107-148.

Morel. (1968). La guérison des pommes de terres atteintes de maladies à virus.Ann Physiol. Veg. 10:113–139.

Schwartz D (1993). Méthodes statistiques à l'usage des médecins et biologistes. Edité par Flammarion medecine-sciences.

Zryd (1988). Cultures de cellules, tissus et organes végétaux. Presses polytechniques Romandes.